UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 15 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: EEB review of two acute toxicity studies with DBNPA (biobrom technical).

From: Jim Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division, (H7507 C)

To: Walter Francis, Product Manager
Barbara Pringle, PM Team 32, Reviewer
Registration Division (H7505 C)

Acute toxicity testing on Mysid shrimp (Accession #416697 -01) and Sheepshead minnow (Accession #416697 -02), have been found to satisfy guideline requirement 72-3 for testing with estuarine crustaceans and fish.

JAN 15 1991

DATA EVALUATION RECORD

1. CHEMICAL: Biobrom

2. TEST MATERIAL: DBNPA (Biobrom C-103), 99.5% a.i. dibromonitrilopropionamide, white crystal, Lot #0073

3. STUDY TYPE: Acute Toxicity Test for Estuarine/Marine Organisms (Estuarine Fish 96-Hour Acute Toxicity Study)

4. CITATION: Sousa, J.V. 1990. (DBNPA) - Acute Toxicity To Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Performing Laboratory; Springborn Laboratories, Inc., Wareham, MA. Study Sponsor; Bromine Compounds Ltd., Beer-Sheva, Israel, Ameribrom Inc. Accession # 416697- 02

5. REVIEWED BY:

Greg Susanke, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)

Greg Susanke 1/3/91

6. APPROVED BY:

Les Touart, Acting Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)

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7. CONCLUSION: This study satisfies Guideline Requirement 72-3 for an Estuarine/Marine Fish Acute Toxicity Study. DBNPA is moderately toxic to estuarine fish. The LC50 = 3.4 ppm, and the 95% C.I. is 3 - 4.9 ppm. The NOEL is .33 ppm.

2

8. MATERIALS AND METHODS:

A. Test Organisms:

Species- Sheepshead minnow (Cyprinodon variegatus)

Supplier- Aquatic Biosystems, Fort Collins, CO.

Mean weight- 0.27 g (0.12 - 0.69 g), wet weight

Mean length- 24 mm (20 - 32 mm)

Acclimation period- Fish were held for a minimum of 14 days in natural seawater at: temperatures of 21 - 22 °C; salinity of 31 - 35 °/oo; pH of 7.4 - 7.5; DO equal to 85 - 97% of saturation; and a photoperiod of 16 hours light and 8 dark. There was 0.4% mortality in the test population during the 48 hours prior to testing. Fish were fed daily, but food was withheld 48 hours prior to test initiation.

B. Test System:

Source of dilution water- Natural seawater was collected from the Cape Cod Canal, Bourne, MA then filtered. Dilution water quality was biologically monitored in the laboratory by the maintenance of continuous mysid cultures.

Water temperature- 23° C as maintained by water bath

pH- 7.8 - 8.1

Dissolved oxygen- 5.8 - 7.3 mg/L (81 - 101% saturation)

Salinity- 30 - 32 °/oo

Total organic carbon- 2.0 mg/l (measured for month of July)

Test aquaria- 8.0 L glass aquaria (29.25 x 14.5 x 19 cm), water volume fluctuation between 3.4 - 5.9 L.

Type of dilution system- Continuous-flow serial diluter with a dilution factor of 60%

Flow rate- 72 L/day or 12.2 volume replacements/ day. This rate replaced 90% of the test solution every 4.5 hours.

Biomass loading rate- .0375 g/L per day

Photoperiod- 16 hours fluorescent light (intensity 30 - 80 footcandles) and 8 hours dark, sudden transitions were avoided.

C. Test Design:

Range finding test- Nominal concentrations of 10, 6.0, 3.6, 2.2, and 1.3 ppm were used. There was 100% mortality at 10 and 6.0 ppm. All fish at 3.6 ppm were observed to be lethargic, but there were no mortalities at this concentration or 2.2 ppm. There was 20% mortality at 1.3 ppm.

Definitive test

Nominal concentrations- 10, 6.0, 3.6, 2.2, 1.3, and 0.78 ppm

Controls- dilution water control

Number of test organisms- 10 sheepshead minnow per replicate (20 per treatment level)

Biological observations- Observations were made at test initiation and at 24 hour intervals.

Water parameter measurements- Temperature, salinity, pH, and DO were measured daily in each replicate of each treatment level and control. Temperature was measured continuously in one replicate of the dilution water control. No insoluble test material was observed in solution.

9. REPORTED RESULTS:

Mean measured concentrations- 7.9, 4.9, 3.0, 1.40, 0.55, and 0.33 ppm as measured at 0-hour and 96-hours. Measured concentrations were 79%, 82%, 83%, 64%, 42%, and 42% of the nominal concentrations, respectively. The coefficient of variation averaged 6% for all mean measured concentrations.

Recovery of chemical- mean recovery was 105% $\pm$ 8.2% of DBNPA from filtered seawater

Mortality and observations- There was 100% mortality at 7.9 and 4.9 ppm, 25% mortality at 3.0 ppm, and 0 mortality at 1.4, 0.55, 0.33 ppm or the control. Lethargic and erratic swimming behavior was observed at 3.0, 1.4 and 0.55 ppm.

4

10. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

"the effects observed during this study were clearly concentration-dependent. The 96-hour LC50 for sheepshead minnow exposed to DBNPA was estimated by non-linear interpolation to be 3.4 mg A.I./L... The No Observed Effect Concentration (NOEC) for sheepshead minnow exposed to DBNPA was determined to be 0.33 mg A.I./L."

11. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: Test procedures were generally in accordance with protocols recommended by the Guidelines. The protocol deviations listed below are not expected to affect the results of the study.

- The mean weight of the fish used in this study was 0.27 g. The recommended weight is 0.5 - 5.0 g.

- Test solution volume ranged from 3.4 - 5.9 L per aquarium. The recommended volume is 15 L or slightly larger. Although the test volume was small, the loading factor is acceptable because the fish used were also small.

- The flow rate was 12.2 volume replacements per day. The recommended rate is 5 to 10.

B. Statistical Analysis: Statistical analysis was performed using the EEB Toxanal Computer Program. The LC50 was determined by the Binomial Method because neither the Probit Method nor the moving average can be used when there are less than two concentrations at which the percent dead is between 0 and 100.

C. Discussion/Results: This study appears to be scientifically sound. The 96-hour LC50 value has been determined to be 3.4 ppm, and the 95% C.I. is 3 to 4.9 ppm. Therefore, DBNPA is moderately toxic to estuarine fish. The NOEL is .33 ppm.

D. Adequacy of the Study:

1. Classification: Core

2. Rationale: N/A

3. Repairability: N/A

Greg Susanke DBNPA Estuarine Fish Acute Toxicity

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.9	20	20	100	9.536742E-05
4.9	20	20	100	9.536742E-05
3	20	5	25	2.069473
1.4	20	0	0	9.536742E-05
.55	20	0	0	9.536742E-05
.33	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3 AND 4.9 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.423269

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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DATA EVALUATION RECORD

1. CHEMICAL: Biobrom

2. TEST MATERIAL: DBNPA (Biobrom C-103), 99.5% a.i. dibromonitrilopropionamide, white crystal, Lot # 0073

3. STUDY TYPE: Acute Toxicity Test for Estuarine/Marine Organisms (Shrimp 96-Hour Acute Toxicity Study)

4. CITATION: Sousa, J.V. 1990. (DBNPA) - Acute Toxicity to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. Performing Laboratory; Springborn Laboratories, Inc., Wareham, MA. Study Sponsor; Bromine Compounds Ltd., Beer-Sheva, Israel, Ameribrom Inc. Accession # 416697- 01

5. REVIEWED BY:

Greg Susanke, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)

Greg Susanke 1/3/91

6. APPROVED BY:

Les Touart, Acting Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)

L. Touart 1/14/91

7. CONCLUSION: This study satisfies Guideline requirement 72-3 for an Estuarine/Marine Acute Toxicity Study with Mysid Shrimp. DBNPA has been found to be highly toxic. The LC50 = 0.72 ppm and the 95% C.I. is 0.52 - 1.3 ppm. The NOEL is 0.23 ppm.

7

8. MATERIALS AND METHODS:

A. Test Organisms:

Species- mysidopsis bahia

Supplier- In house laboratory culture

Age- ≤ 24 hours old

Acclimation period- The mysid culture was maintained at: 25 ± 1° C, 16 hours light (at an intensity of 70 - 110 footcandles) and 8 hours dark, and were fed brine shrimp twice daily (continued through study).

B. Test System:

Source of dilution water- Natural seawater was collected from the Cape Cod Canal, Bourne, MA then filtered. Dilution water quality was biologically monitored in the laboratory by the maintenance of continuous mysid cultures.

Water temperature- 25° C as maintained by water bath

pH- 7.9 - 8.1

Dissolved oxygen- 5.9 - 6.6 (86 - 96% saturation)

Salinity- 31 - 32 °/oo

Total organic carbon- 2.2 mg/L (measured monthly)

Test aquaria- 8 L (29.25 x 14.5 x 19 cm, water volume fluctuated between 3.4 and 5.9 L.

Type of dilution system- Continuous flow serial diluter with dilution factor of 60%

Flow rate- 72 L/day or 12.2 volume replacements/day. This rate allowed test solution to be replaced at 90% every 4.5 hours.

Biomass loading rate- < 3 mg/L

Photoperiod- 16 hours fluorescent light (intensity 32-80 footcandles) and 8 hours dark, sudden transitions were avoided

8

C. Test Design:

Range finding test- Nominal concentrations of 5.0, 3.0, 1.8, 1.1, 0.65 and 0.39 ppm were used. There was 100% mortality at the three highest treatment levels and 0, 10 and 20% mortality at 1.1, 0.65, and 0.39 ppm, respectively.

Definitive test

Nominal concentrations- 3.0, 1.8, 1.1, 0.65, 0.39, and 0.23 ppm

Controls- dilution water control

Number of test organisms- 20 mysids per treatment level and control, 10 per replicate (five per retention chamber)

Biological observations- Observations were made at test initiation and subsequent 24 hour intervals.

Water parameter measurements- Temperature, salinity, DO, and pH were measured daily in each replicate of the control and treatment levels. Temperature was measured continuously in one replicate of the dilution water control. NO precipitate or insoluble test material was observed.

9. REPORTED RESULTS:

Mean measured concentrations- 2.4, 1.3, 0.52, 0.23, and 0.11 ppm. Measured concentrations were 80%, 72%, 47%, 35%, and 28% of nominal concentrations, respectively. The measured concentration of the lowest nominal concentration was below the detection limit of 0.067 ppm. Therefore, it was not included in the LC50 calculation.

Recovery of chemical- average recovery was 105% (± 8.2) from filtered seawater

Mortality and observations- There was 100% mortality at 2.4 and 1.3 ppm. There was 15% mortality at 0.52 ppm and all surviving mysids were lethargic. No mortalities or sublethal effects were observed at 0.23 or 0.11 ppm.

10. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

The 96-hour LC50 value was calculated to be 0.72 ppm by nonlinear interpolation, and the 95% C.I. is 0.52 - 1.3 ppm. The No Observed Effect Concentration was 0.23 ppm.

7

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

11. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: Test procedures were generally in accordance with protocols recommended by the Guidelines. The protocol deviations listed below are not expected to affect the results of the study.

- The flow rate was 12 volume additions per 24 hours. The recommended amount is 6 - 10.

- Water temperature was 25° C, but the recommended rate is at or around 22° C.

B. Statistical Analysis: Statistical Analysis was performed using the EEB Toxanal Computer Program. The LC50 was determined by the Binomial Method.

C. Discussion/Results: This study appears to be scientifically sound. The 96-hour LC50 has been determined to be .72 ppm, and the 95% C.I. is .52 and 1.3 ppm. Therefore, DBNPA is highly toxic to estuarine/marine crustaceans. The NOEL is 0.23 ppm.

D. Adequacy of the Study:

1. Classification: core

2. Rationale: N/A

3. Repairability: N/A

12. COMPLETION OF ONE-LINER FOR STUDY: yes

10

Greg Susanke DBNPA (Biobrom) Mysid Shrimp LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.4	20	20	100	9.536742E-05
1.3	20	20	100	9.536742E-05
.52	20	3	15	.1288414
.23	20	0	0	9.536742E-05
.11	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .52 AND 1.3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .7174721

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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